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HEAT-INDUCED CHANGES IN PERFUSED RAT LIVER, (U)

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## **Heat-Induced Changes in Perfused Rat Liver**

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**Running head: Heat load and structural change in liver**

**Key words: Hyperthermia, perfused liver, electron microscopy, bile,  
transaminases**

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### Abstract

In order to determine the time periods at which heat-induced structural changes occurred, isolated rat livers were perfused for different time intervals at 41° - 43°C with oxygenated Krebs-Ringer bicarbonate containing .14% glucose. Structural changes occurred after 60 minutes at 41°C, 30-45 minutes at 42°C and 15 minutes at 43°C. Under these perfusion conditions, structural changes were detected before bile production or enzyme leakage reflected changes. The data suggest a continuum of events depending on the time and temperature of exposure, leading to the same end point: loss of endothelium, vacuolization, loss of microvilli, and formation of flocculent densities in mitochondria.

The effects of heat on the liver have been investigated in several laboratories (1,2,3,4,5,6,7). Hepatic injury resulting from heatstroke (1,6,7,8) or occurring with thermotherapy for cancer (9) has stimulated the use of the isolated perfused liver as a model system for studying the direct effects of heat on this organ (3,4,10). We previously described the structural changes occurring in rat livers perfused for 90 minutes at  $37^{\circ} - 43^{\circ}\text{C}$  (3), and significant structural damage was observed at  $41^{\circ} - 43^{\circ}\text{C}$ . These histological and ultrastructural findings were supported by data from other investigators utilizing the perfused liver and different parameters as indications of injury (4,10). Since tissue specimens were not taken until the end of the 90 minute heating episode (3), the actual time of onset of structural damage was not determined; however, data on the leakage of transaminases and bile production indicated that some changes occurred during shorter periods of hyperthermia.

The purpose of this study was to identify the time periods at which structural changes occurred in isolated rat livers, which were perfused with oxygenated Krebs-Ringer bicarbonate (KRB) at temperatures from  $41^{\circ} - 43^{\circ}\text{C}$ , and relate these to changes in transaminase leakage and bile production.

#### Methods

The procedures for isolation and perfusion of rat livers were similar to those described by Miller (11) and Bowers et al. (3). Livers were isolated from male Sprague-Dawley (Charles River) rats with a mean weight of  $438 \pm 49$  g. Livers were perfused for 90 minutes, 75 minutes, 60 minutes, 45 minutes, 30 minutes or 15 minutes at  $41^{\circ}$ ,  $42^{\circ}$  or  $43^{\circ}\text{C}$ .

Each exposure time was represented by a group of 8 perfused livers. In order to minimize the number of livers required, the 90 minute group for each temperature was perfused first, followed by the 75 minute, 60 minute, and so forth until a group of livers was reached which had no significant structural lesions. At 41°C, this point was reached with 45 minute exposures. Therefore only 4 time groups were studied at this temperature (4 groups x 8 livers/group = 32 livers). At 42° and 43° all six groups were studied (n = 48 at each temperature). Eight control livers were perfused for 90 minutes at 37°C.

The perfusate consisted of Krebs-Ringer bicarbonate which was oxygenated with humidified gas (90 - 95% oxygen with the balance CO<sub>2</sub>) and contained 0.14% glucose. A flow rate of 40 ml per minute was maintained to insure adequate oxygen supply (12,13) and the perfusate was not recirculated. Samples of perfusate exiting the livers were taken at 0 time and every 15 minutes until perfusions were terminated. These samples were analyzed for levels of alanine aminotransferase (GPT) and aspartate aminotransferase (GOT) using a Gilford 3402 automatic enzyme/end point analyzer and Worthington/Gilford Statzyme kits. Bile was collected in 1 cc tuberculin syringes with sealed tips and the volume was noted every 15 minutes. At the end of the perfusion for each group, specimens were taken from the median lobes, fixed with neutral buffered formalin and processed for light microscopy using hematoxylin and eosin (H and E) stain. Similar specimens were fixed with 2% glutaraldehyde in cacodylate-sucrose buffer, followed by 1% OsO<sub>4</sub> in the same buffer. These were embedded in Epon, sectioned and stained with uranyl acetate and lead citrate, and examined with a JEOL 100B electron microscope. Dunnett's multiple comparison test (14) was used to determine statistical significance (p < .05) when comparing enzyme levels and bile.



## Results

Control livers perfused at 37°C for 90 minutes produced bile at a constant rate (Fig. 1-A), had little or no detectable enzyme leakage (Fig. 1-B and C), and had normal hepatic structure. Bile production at 41°C (Fig. 1-A) was not significantly different from control values until 60 minutes (Table 1 shows statistical significance for bile readings and enzyme leakage). Increasing levels of enzymes were evident in perfusates exiting the livers after 45 minutes (Figs. 1-B and C), but these were never statistically significant at 41°C. Light microscopy (Fig. 2-A) and electron microscopy (Fig. 2-B) indicated normal structure for seven of eight livers at 45 minutes; however, these structural parameters showed focal lesions in all eight livers perfused for 60 minutes at 41°C. Focal hepatocellular necrosis, loss of endothelium, reduction in hepatocyte microvilli and evidence of cell debris in sinusoids were characteristic lesions (Figs. 3-A and B).

At 42°C, bile production by the 45 minute group was significantly different from that of controls, but that of the 75 minute group was not. This reflects an inconsistency in this parameter. GPT and GOT levels increased after 45 minutes (Figs. 1-B and C) but were not significantly different from control values until 60 minutes at this temperature. Light and electron microscopy indicated normal structure at 15 minutes, but three of eight livers showed vacuolization after 30 minutes and focal centrilobular vacuolization was present in all eight livers after 45 minutes (Fig. 4-A). Note the presence of flocculent dense bodies in mitochondria (Fig. 4-B).

At 43°C bile production was not significantly different from control values until 75 minutes. GPT leakage was significantly

different from control values at 60 minutes, while GOT levels were significant at 45 minutes. Half of the livers perfused at 43°C for 15 minutes showed normal structure while the other half showed areas of focal vacuolization (Fig. 5-A) accompanied by loss of endothelial cells and the appearance of hepatocellular debris in the sinusoids (Fig. 5-B).

Hepatic injury increased in frequency and severity with both exposure time and heating intensity. The distribution of cells containing flocculent densities in mitochondria (Fig. 4-B) was widespread at longer time intervals (90 minutes) and higher temperatures (42° and 43°C) but such cells were only occasionally observed at lower heat loads.

#### Discussion

These experiments were designed to detect the earliest time at which structural damage occurred, and also to determine how structural changes related to changes in bile production and enzyme leakage during exposure to heat.

The first two bile readings (15-30 minutes) were good indicators of the success of the perfusions. Problems with the perfusate or surgery produced 15 and 30 minute readings which were low, and the 30 minute readings determined whether livers were rejected or the perfusion continued. Later volume readings in the heated groups indicated that bile production was a labile phenomenon, under severe conditions, which varied to some extent with factors other than heat exposure. Thus, bile production was not a good indicator of the time at which detectable structural change occurred. Data on bile production in experiments by John and Miller (15) suggest that a more complex perfusate might

eliminate this problem. In their study, bile production by control livers was best when the perfusate contained RBC, amino acids, insulin and cortisol.

The leakage of transaminases (GPT and GOT) into the perfusate was more consistent. At 41°C these values were never statistically different from controls. Statistical significance was achieved at 60 minutes for both GPT and GOT at 42°C, while at 43°C significant elevations occurred at 60 minutes (GPT) and 45 minutes (GOT). It was anticipated that minor alterations in membrane permeability would produce significant enzyme leakage prior to the detection of pronounced structural changes. The fact that statistically significant increases did not occur at 41°C and occurred after periods when structural changes were observed at 42°C and 43°C probably related to the 40 ml/minute flow rate and the single pass system. If the perfusate were recirculated or the flow rate were reduced, the same rate of enzyme leakage would produce higher values.

The exposure time required to produce structural damage was 60 minutes at 41°C, 30-45 minutes at 42°C and 15-30 minutes at 43°C. These structural changes confirmed previous observations that heat-induced injury to the isolated liver perfused with KRB occurred at 41°C (3,4,10); however, there is sufficient evidence to indicate that exposure to this temperature represents a transitional zone and probably reversible injury with short exposure times. The structural changes which occurred after longer perfusion times (90 minutes) were identical to those described previously (3). Although exposure to 41°C appeared to represent a transitional zone, the early vacuolization which occurred at 42°C and 43°C was similar to that which occurred later at 41°C. It is

evident that both the time and temperature of exposure (heat load), not just the specific temperature of exposure, determine when injury occurs and the extent of damage in the isolated organ between 41°C and 43°C. This is important because it verifies the concept in a system independent of the usual coincidental changes in vivo due to dehydration, perfusion pressure, acid-base balance, substrate availability and hormone flux. Of the three major hypotheses for the mechanism(s) of heat-induced injury (changes in membrane permeability, release of lysosomal enzymes, and damage to proteins), the hydropic changes resulting in vacuolization and leakage of transaminases lend support to the hypothesis that heat induces changes in membrane permeability. Of course, damage to membrane proteins could be involved in this process as well.

Although Wills et al. (9) suggested that some degree of liver damage probably occurs in all patients treated with hyperthermia, it is not clear why hepatic injury routinely occurs at 41°C in isolated perfused livers (3,4,10), while most studies of intentional hyperthermia in humans indicate that no heat induced hepatic damage occurs at 42°C with even longer exposures (16). This may reflect the presence of hepatoprotective humoral factors in the latter group, or lower sensitivity in monitoring hepatic injury in vivo. The use of a more complex perfusate and a closed recirculating system (15) should further refine the timing of heat-induced events and could result in the identification of hepatoprotective humoral factors.

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TABLE 1

Summary of statistical comparison of control and experimental data  
for bile, GPT and GOT.

	<u>Bile</u>			<u>GPT</u>			<u>GOT</u>		
	41°	42°	43°	41°	42°	43°	41°	42°	43°
30 min. group	-	-	-	-	-	-	-	-	-
45 min. group	-	+	-	-	-	-	-	-	+
60 min. group	+	+	-	-	+	+	-	+	+
75 min. group	+	-	+	-	+	+	-	+	+
90 min. group	+	+	+	-	+	+	-	+	+

- not significantly different from control group perfused at 37°C (Dunnett's Multiple Comparison Test,  $p < .05$ ).

+ significantly different from control values.

### Figure Legends

Figure 1. (A) Bile accumulated at the time of termination of perfusions is indicated. Each bar represents a different group of eight livers perfused at 37°C, 41°C, 42°C, or 43°C. Markers indicate standard error of the mean (SEM). (B) GPT level in the perfusate at the time of termination of perfusions. (C) GOT level in the perfusate at the time of termination of perfusion.

Figure 2. (A) Light micrograph showing normal structure in rat liver after 45 minutes at 41°C, 240 X. (B) Electron micrograph showing normal structure in rat liver perfused for 45 minutes at 41°C, 10,000 X.

Figure 3. (A) Light micrograph showing periportal damage after perfusion at 41°C for 60 minutes, 240 X. (B) Electron micrograph showing ultrastructural changes after 60 minutes at 41°C, 10,000 X.

Figure 4. (A) Light micrograph showing centrilobular vacuolization in rat liver perfused for 45 minutes at 42°C, 240 X. (B) Electron micrograph showing vacuolization and presence of flocculent dense bodies in mitochondria of rat liver perfused for 45 minutes at 42°C, 10,000 X.

Figure 5. (A) Light micrograph showing centrilobular vacuolization present in four of eight rat livers perfused for 15 minutes at 43°C, 240 X. (B) Electron micrograph showing hepatocellular necrosis occurring in half the rat liver perfused for 15 minutes at 43°C, 10,000 X.

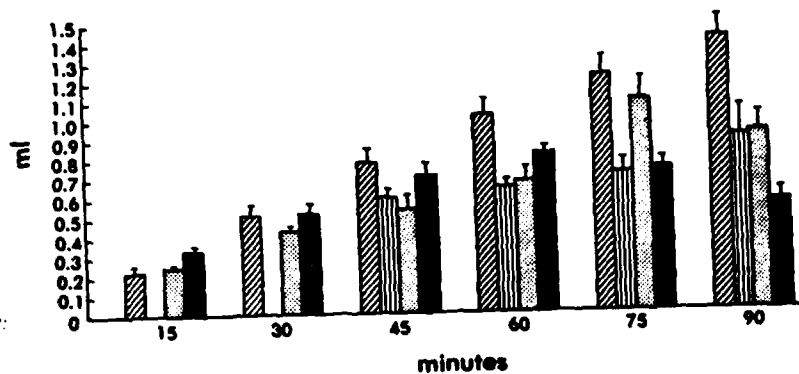


# BILE PRODUCTION

37° 41° 42° 43°

▨ ▩ ▪ ▫

1 A

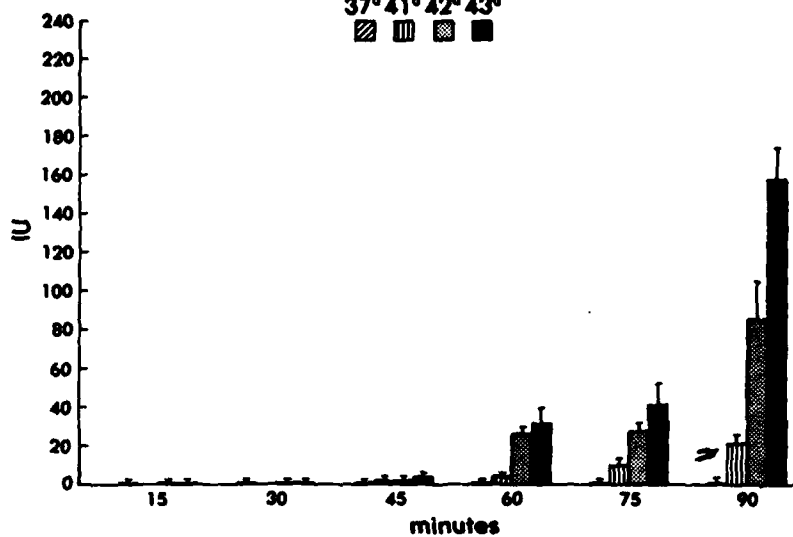


## GPT

37° 41° 42° 43°

▨ ▩ ▪ ▫

B

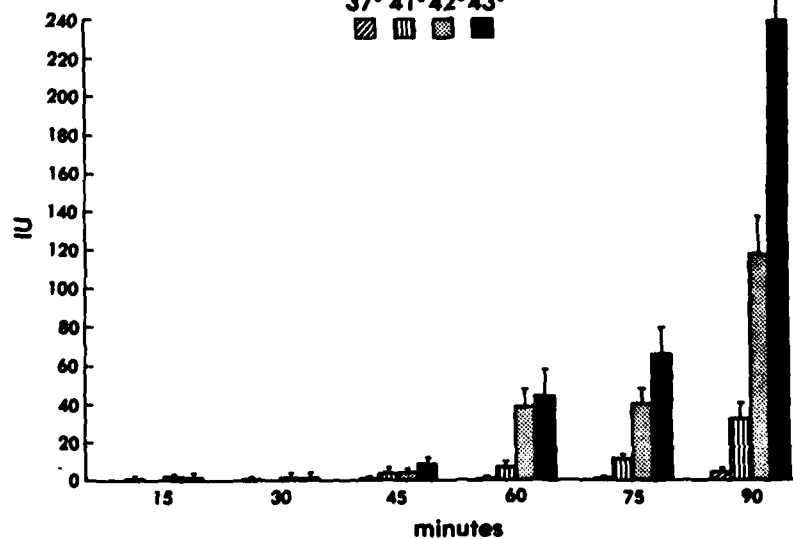


## GOT

37° 41° 42° 43°

▨ ▩ ▪ ▫

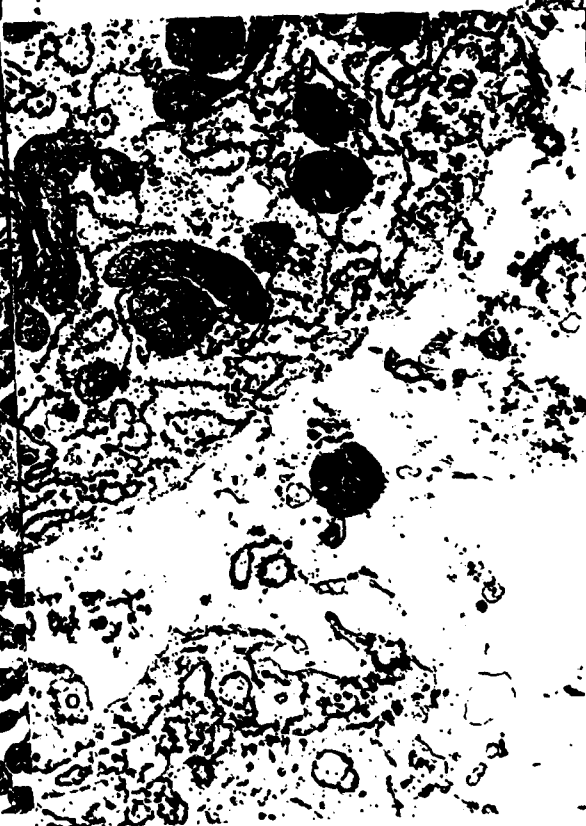
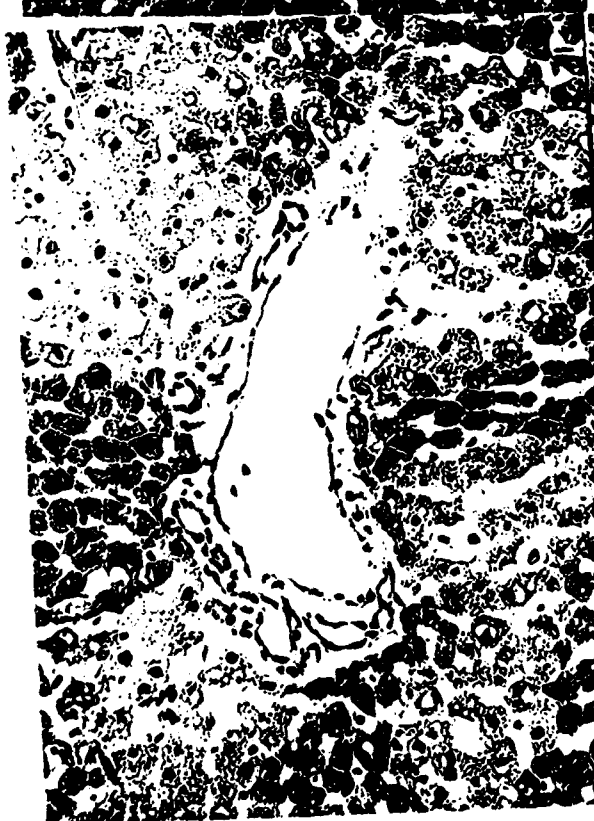
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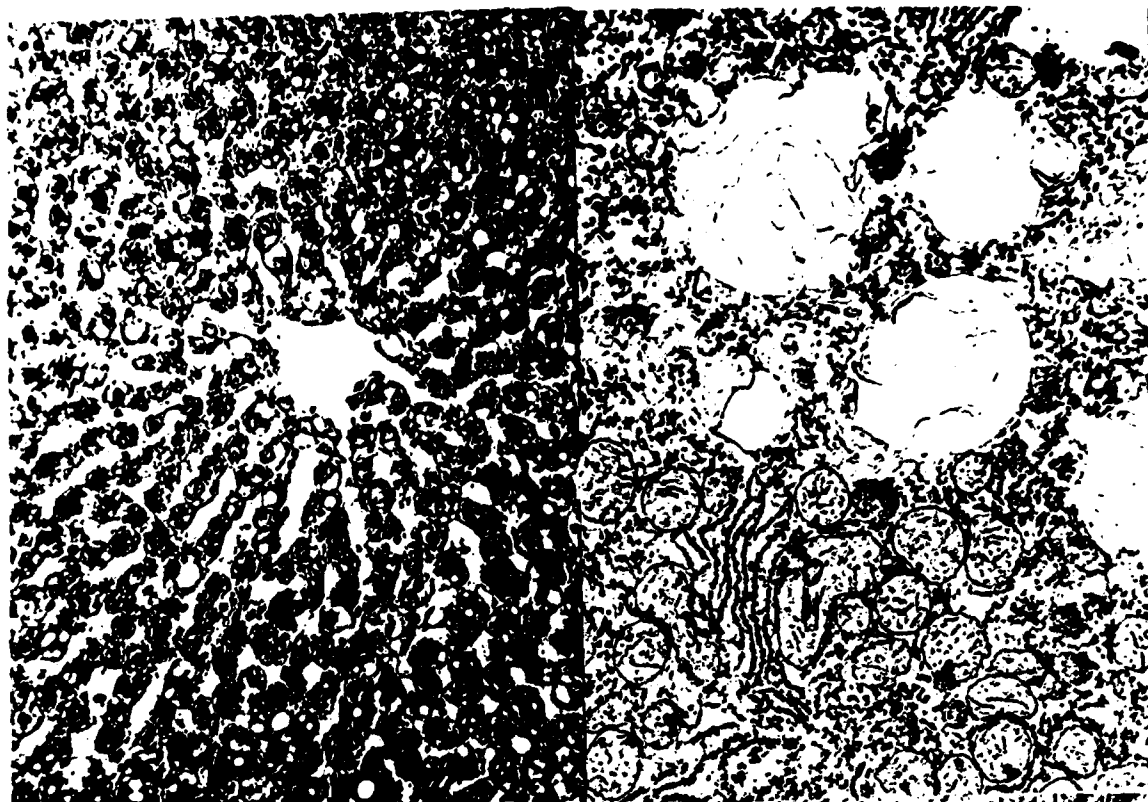
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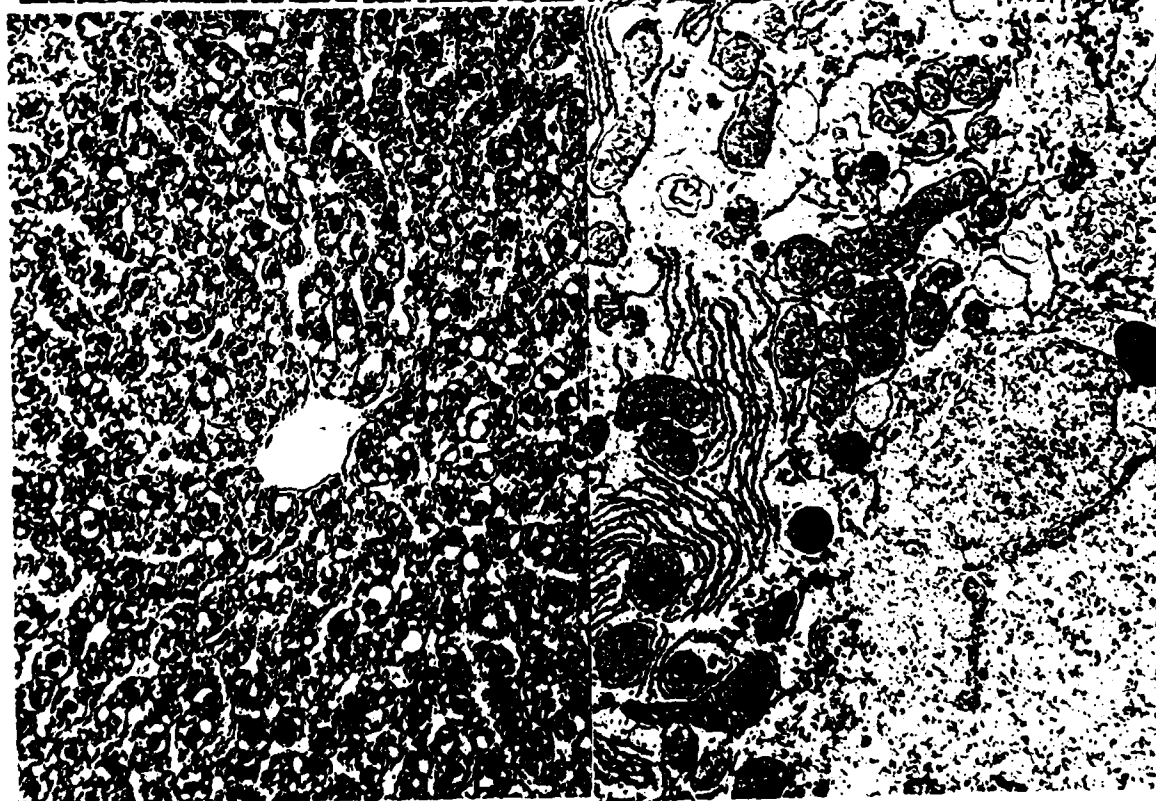
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4A,B



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